

A flipping permease

Lipid bilayers should be a no-flipping zone for integral membrane proteins, with the hydrophobicity blocking any cross-membrane excursions. But now Mikhail Bogdanov, Phillip Heacock, and William Dowhan (University of Texas, Houston, TX) report that fully synthesized lactose permease (LacY) can reverse orientation when the lipid composition of the membrane is changed.

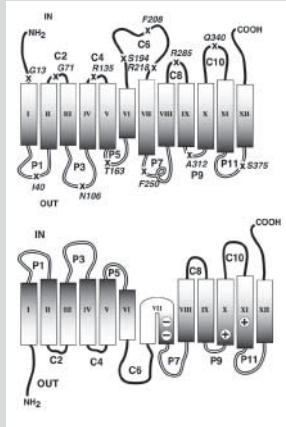
Dowhan focused on phosphatidylethanolamine (PE), which is the only zwitterionic lipid in *Escherichia coli* (the other lipids all have anionic head groups). He knew that PE was needed for LacY activity in vitro, and found that the same was true in vivo. Mutant *E. coli* lacking PE made a version of LacY that allowed facilitated but not active transport of lactose. This aberrant LacY had half of its 12 transmembrane domains in a configuration opposite to that of normal LacY. But when PE synthesis was induced in the absence of new LacY synthesis, the existing LacY flipped back into

its native conformation, and could now do active transport.

The key to this change may lie in the weakly hydrophobic seventh transmembrane domain, which may flip out of the membrane or form a hairpin loop. Dowhan hopes to detect the direction of propagation of the change—where the flipping begins and ends—by blocking the process with a single large covalent modifier. He will also test whether the flipping requires reentry into the translocon machinery.

For LacY, the dependence on PE may be a structural accident. But other proteins function only in certain parts of the cell because of differing lipid environments. And for Dowhan, the result emphasizes the importance of lipids. “Everyone focuses on the proteins, and ignores the lipids, viewing them as a simple solvent,” he says. “But membrane protein sequences are written for a particular lipid environment.” ■

Reference: Bogdanov, M., et al. 2002. *EMBO J.* 21: 2107–2116.



LacY (top) can flip orientations when deprived of a lipid (bottom).

Dowhan/EMBO

Attack of the microvesicles

Melanoma cells send out microvesicles loaded with Fas ligand (FasL) to kill their would-be assassins, according to Stefano Fais (Istituto Superiore di Sanità, Rome, Italy), Licia Rivoltini (Istituto Nazionale dei Tumori, Milan, Italy), and colleagues.

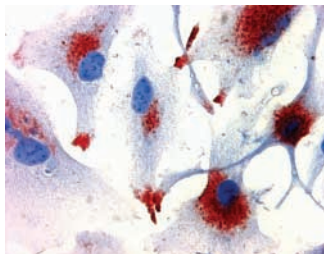
The FasL hijacks a normal transport pathway for melanin, the pigment that melanosomes load into microvesicles and send to neighboring keratinocytes. In melanoma cells, these microvesicles, or exosomes, are also loaded with FasL, which can trigger apoptosis in Fas-expressing immune cells that might otherwise counteract tumor growth.

This mechanism of counterattack may operate in other tumors, as microvesicles are released from a number of cell types. In the case of melanoma cells, the new work clears up a controversy. Microvesicles explain how melanoma cells can have an apoptotic effect (the initial observation) without expressing FasL on their cell surface (the subsequent, seemingly contradictory observation).

Although purified microvesicles can kill cultured Fas-expressing cells, the same cells are not killed by coculture with melanoma cells. This suggests that, in vivo, some other microenvironmental factors may either provoke a higher level of microvesicle production or optimize the effects of the killer exosomes.

Fais also hopes to determine how the Fas-expressing melanoma cells manage to avoid killing themselves with their own microvesicles. ■

Reference: Andreola, G., et al. 2002. *J. Exp. Med.* 195:1303–1316.



Melanoma cells can attack using microvesicles loaded with FasL (red).

Fais/RUP

The transcription–export link

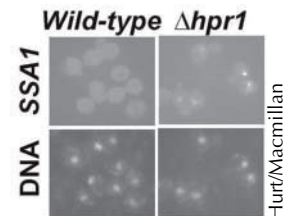
Transcription is a congested business, with many different proteins crowding in to exert their influence. Now Katja Sträßer, Ed Hurt (University of Heidelberg, Germany), and colleagues have found that even nuclear export proteins get into the act, via an interaction with transcription elongation factors. The findings suggest that transcription, splicing and nuclear export are linked processes, and that there are many levels of regulation between these processes that remain to be discovered.

Hurt found that yeast protein Sub2, which with Yra1 connects directly to mRNA exporters, binds a collection of proteins that make up the THO complex. This complex promotes transcription by acting on the elongation step.

The proposed connections were confirmed by synthetic lethal interactions, and with human homologues of the THO proteins. Hurt and colleagues named the combined complex the transcription–export or TREX complex, and found that proteins in the complex could be tracked by chromatin immunoprecipitation as they moved along a transcribed gene with RNA polymerase.

Mutants lacking a THO component had an mRNA export defect, but Hurt does not think that transcripts get posted directly from a gene into a nuclear pore complex. Transcription occurs throughout the nucleoplasm, and TREX factor binding at these sites may simply allow nuclear pores to capture mRNA–protein complexes after they diffuse away from these sites. ■

Reference: Sträßer, K., et al. 2002. *Nature*. 10.1038/nature746.



Defects in a transcription elongation factor lead to problems in mRNA export (right).

Hurt/Macmillan